



**Saskatchewan
Research Council**

**THE POTENTIAL OF USING HONEY BEES AS A
BIOGEOCHEMICAL EXPLORATION TOOL IN NORTHERN SASKATCHEWAN**

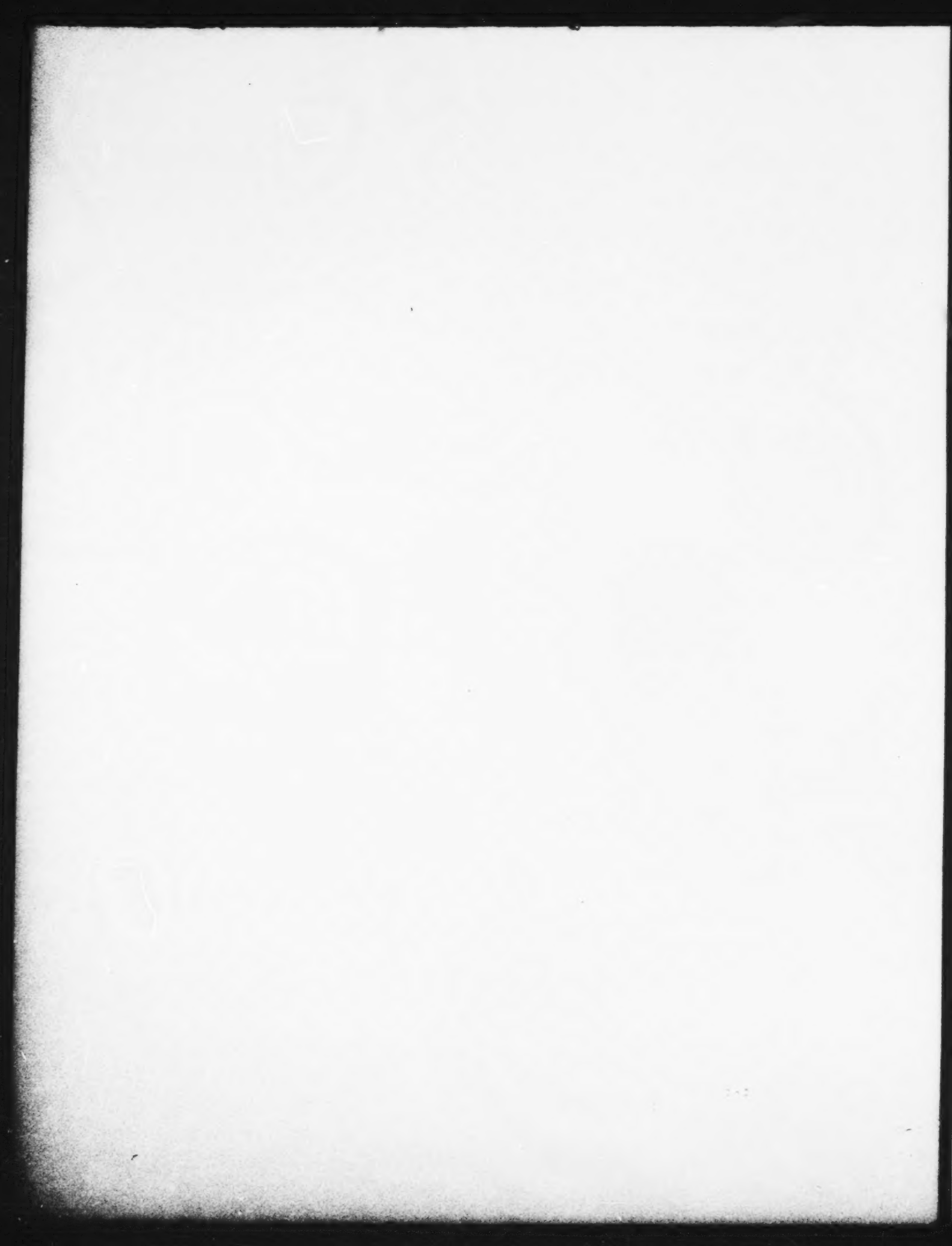
by

**K. Wallace
R. Jähren**

SRC Technical Report No. 185

Publication E-908-6-B-86

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SUMMARY

This preliminary investigation has produced baseline data for 31 elements in bee pollen. In June and July, 1985, beehives were established at two sites in northern Saskatchewan: one at a control "background" site, Gordon Lake (GL); the other near the old Anglo-Rouyn (AR) copper mine, where minor associated gold is present. Soil collected in each area showed the expected higher levels of most elements from AR. Pollen showed the same trend: 18 of the elements were higher at AR, six were below background and four were higher at GL. Three were the same for each site.

The forage area of bees in this environment may be 100 times larger than the area of an individual biogeochemical gold anomaly, giving rise to a great dilution of any gold-enriched pollen by pollen from background areas. Furthermore, gold concentrations vary from one plant species to the next, and since bees visit all flowering species an additional dilution of gold-rich pollen is introduced.

No distinct seasonal variation in pollen chemistry could be discerned, due to the limited number of collections. Variations in chemistry are probably attributable mainly to variations in the species of plant visited by bees during the test period.

ACKNOWLEDGEMENTS

The authors would like to thank the following people:
Dr. Colin Dunn of the Geological Survey of Canada for valued comments and review of this manuscript. Mr. Sid Robinson of La Ronge and Mr. Cliff Buettner of Pinehouse both assisted with pollen collections. Mr. Ian McMurchy (Wildlife Branch) helped out with a loan of electric fencing material.

The study involved help from the following Saskatchewan Research Council staff: J. Ross and R. Begrand for laboratory preparations; J. Campbell and M. Mellinger for geological interpretation; E. Wheaton, S. Shewchuk and J. Maybank for reviewing the manuscript; Z. Abouguendia and J. Hudson for plant species identification; W. Smith for aerial photos; and R. MacPherson for aerial service. The help of all the above is gratefully acknowledged.

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INTRODUCTION

During the past several years much interest has been shown in gold exploration in the La Ronge volcanic belt area of Saskatchewan, and several significant gold discoveries have been made. The present study was undertaken to investigate alternate exploration techniques for gold prospecting. It is known that plants absorb and transport various elements, including gold, from the soil; an example of this is Phacelia.

Girling and Peterson (1978) showed that Phacelia tended to concentrate gold in the tips of the plant. This family of plants exists in northern Saskatchewan. The hypothesis was that it and other flowering species might tend to absorb and translocate gold and other elements to the pollen in a similar way.

Free et al. (1983) showed that the metal content of pollen collected by honey bees was high in areas where the soil was metal-rich. The manganese, zinc, copper, and lead contents of pollen collected from old sites of copper and lead mines were higher than in a control area where no mining took place.

Warren and Horsky (1984) collected bee pollen from plants growing in mineralized and unmineralized areas of British Columbia. The pollen from the mineralized sites had much higher concentrations of Zn, Cu, Pb, Mo, Cd, Fe, Mn and As than pollen from unmineralized areas. Mo values were up

to 100 times higher in the mineralized area.

Biogeochemical exploration for minerals makes use of flowering plants as well as trees. Preliminary investigations in northern Saskatchewan (Dunn, 1984) indicated that gold concentrations in ashed vegetation were highest for alder twigs (Alnus crispa), about the same for jack pine (Pinus banksiana) and balsam fir (Abies balsamifera) twigs, and lowest for black spruce (Picea mariana) and balsam fir bark. He also found a striking variation in seasonal changes in gold content of ashed alder twigs. The mean gold content of individual shrubs from fourteen locations was 28 ppb in June, yet only 10 ppb in August. Arsenic and barium behaved in a similar fashion but with smaller seasonal differences. Later work (Dunn, 1985) showed that the highest concentrations appear in early spring (mid April 1985 - 69 ppb Au). He also reported that alders did not give similar gold levels from 1984 to 1985. He ascribes the differences to climate variation because over the year the tree nutrients are mobile at different dates.

The process of collecting alder twigs, leaves and outer bark scales of spruce and jack pine is labour intensive. The present preliminary study was undertaken to evaluate the potential for using honey bee pollen as an indicator of metal uptake from flowering species. An advantage of this technique could be lower labour costs than for conventional biogeochemical methods.

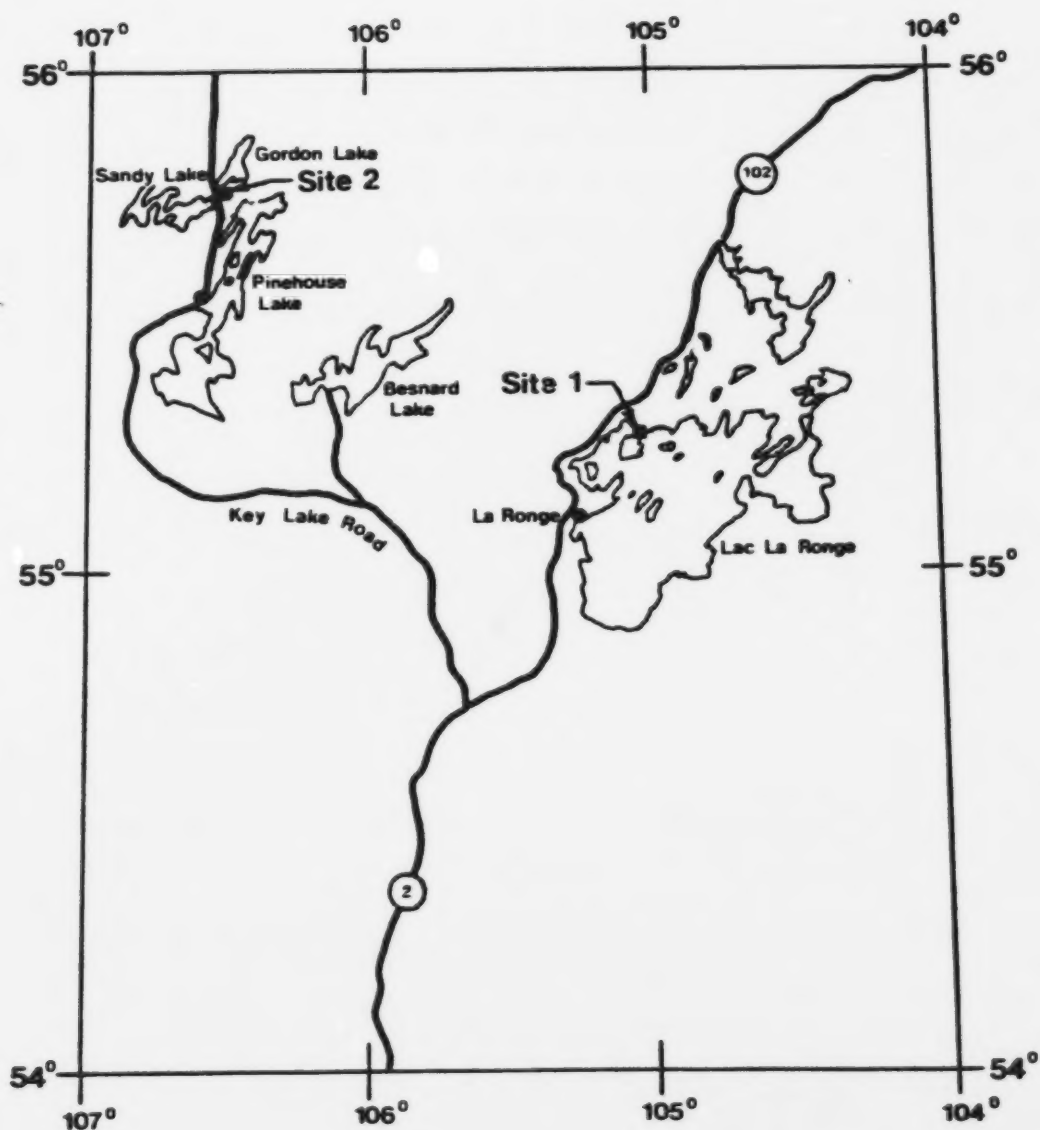
The forage range of honey bees extends to 4 km and possibly further, depending on the availability of flowering species (Free et al., 1983). The forage area would be approximately 50 km². During each day a foraging bee makes about 10 trips visiting about 1000 flowers each trip. The bees collect pollen on the corbiculae on their rear legs. Thousands of plants of several species are visited each day. It is important to note that pollen from different plants is not mixed on each forage trip. The pollen from one species is collected on that particular trip only.

FIELD MATERIALS AND METHODS

Four packages of honey bees were obtained from California in early May. They were kept in Saskatoon in standard beekeepers' hives (one super) until transported to the north on June 4th. Ontario Agriculture College (OAC) (bottom trap) pollen traps were installed, to form the entrance to each hive. These traps scrape the pollen from the rear legs of the bees as they enter the hive.

In early June two honey bee colonies were situated at each of the two sites; 1) the southern La Ronge volcanic belt at the old Anglo-Rouyn (AR) copper mine where minor gold mineralization is known; and 2) in an area suspected to contain no mineralization (Wathaman-Batholith) approximately 28 km north of Pinehouse Lake (see Figure 1). At the second site the colonies were located 200 m south of Gordon Lake (GL) in a clear area where gravel had been mined and stock-piled. Prior to selecting the latter site other areas were investigated along the Key Lake road but lack of flowering species and excessive amounts of dust due to truck traffic precluded their use. The dates of pollen collection, color and approximate quantity of pollen during each period were recorded.

One hive at the AR site did fail; apparently the queen from the other hive was much stronger, as very little activity or pollen was found after the second visit on July 28th. Electrified bear-proof fencing was erected around the hives,



UTM Coordinates

Site 1 (AR) Anglo-Rouyn Mine: 500,000 mE , 6,127,700 mN

Site 2 (GL) Gordon Lake: 402,700 mE , 6,181,900 mN

Figure 1 Study Area Site Locations.

and canned sardines were hung from the fencing to ensure that wildlife obtained a sufficient electrical charge to deter them.

Soil samples were taken around the hives at each site in four directions at distances ranging from 0.75 to 3 km. The samples were composited into a top organic layer which consisted of peat and humus (L-H) horizon. The bottom layer usually consisted of sand although some samples were till, generally A_C over $B_{m,f}$ horizons.

Bee pollen was collected during June and July for various lengths of time. The first collection from the AR site indicated pollen was being brought in at a very high rate. It was felt the hive entrance could become blocked due to the high pollen accumulation if left longer than a few days. The pollen traps were not installed on a continuous basis so that some pollen was allowed into the hive for food.

The pollen production dropped off at both sites over the period June to July. Presumably there was a decrease in early flowering species, but many of the northern flowers blossom late and were not caught by our collection period. Another factor governing pollen collection is the number of sunshine hours available, as bees navigate by the sun. On cloudy or rainy days the bees do not venture out any significant distance from the hive. The thirty year normal (1951-1980) sunshine hours for Cree Lake show a 9% reduction from Saskatoon values for June and a 18% decrease for July (En-

vironnement Canada, 1982). The pollen collected from northern regions would be reduced compared to Saskatoon.

SAMPLE ANALYSIS AND RESULTS

The soil samples were air dried and mixed thoroughly. The top layer, consisting of organic components (peat and humus L-H horizon), was ground to ensure proper mixing. The bottom layer was predominantly from the A_C horizon with minor amounts from the B_{m,f} horizon. The compositing produced four samples in total representing the top and bottom portions from each site. The soil was dried at 105°C for two hours and 10 g was weighed into vials for Neutron Activation Analysis (NAA).

Bee pollen samples were collected according to the schedule in Table 1. The pollen samples were transported in sealed polyethylene bottles and refrigerated at the Saskatchewan Research Council. Prior to analysis the seven bulk pollen samples were separated into green, yellow and miscellaneous colors. The need for the miscellaneous category arose because only one or two pollen samples showed the colors brown, black, orange or beige. The samples were oven dried at 105°C for two hours and a minimum of 8 g was weighed into plastic vials for analysis. The moisture content of the bulk pollen varied from 12-22%.

Portions of the samples were ashed at 570°C for two hours in crucibles. The ash content ranged from 2.5 to 5.5%, being twice as high from AR compared to GL. These results are similar to those found by Todd (1942) when he analyzed 34 pollen samples from California. He found the

Table 1. Pollen Collection Data.

Sample Identification	Collection Dates	Duration (Days)	Predominate Color	Quantity Collected*
GORDON LAKE SITE (GL)				
PC-1	June 5-11	7	yellow green	heavy
PG-2	July 2-5	4	yellow	medium
PG-3	July 19-24	6	green	light
ANGLO ROUYN SITE (AR)				
PAR-1	June 4-6	3	yellow green	heavy
PAR-2	June 24-28	5	yellow	medium
PAR-3	July 11-15	5	yellow	light
PAR-4	July 15-22	8	green	light

* Light <25 g, medium 25-60 g, heavy >60 g; weights recorded prior to drying.

average composition of ash to be: potassium 20.7%, phosphorus 13.6%, calcium 10.5%, magnesium 6.7% and iron 0.07%.

The dry samples were analyzed by neutron activation analysis for the standard biogeochemical suite of 31 elements offered by Nuclear Activation Services Ltd. The technique is highly sensitive and nondestructive. Pollen samples were not ashed prior to analysis due to the volatility of some of the elements, particularly Se, Br, Cs, As, Rb and Fe (Dunn, 1985). The soil samples (Table 2) from the AR site, particularly the top organic portion have higher concentrations of most elements than at the GL site. The same trend is evident for the bottom soil/sand layer. This establishes the higher concentration of elements potentially available to plants at the AR location.

Most elements in unashed pollen samples were present in very low concentrations, with the exception of major elements Fe, Ca and Na. There were insufficient pollen collections at each site to perform a complete statistical evaluation of the data. The elements Au, Br, Ca, Co, Fe, La, Na, Rb, Sm and Zn consistently displayed high enough concentrations for a correspondence analysis (factor analysis method) to be performed. The technique used on the data is more descriptive than statistical, but it allows easy visualization of the data set on a bivariate plot. The results of the analysis suggest the variability within each site is as important as that between sites. The AR and GL sites have

Table 2. Element Concentrations in Dried Soil (Determined by Neutron Activation Analysis).

Element	Detection Limits	Concentration Range			
		Anglo Rouyn Site n=13		Gordon Lake Site n=10	
		Top*	Bottom**	Top*	Bottom**
Ag	5 ppm	<5	<5	<5	<5
As	2 ppm	4	5	<2	<2
Au	5 ppb	110	19	<5	<5
Ba	100 ppm	400	600	400	400
Ca	0.5 %	1.8	1.7	<0.5	<0.5
Co	5 ppm	20	9	<5	<5
Cr	10 ppm	40	40	10	10
Fe	0.02 %	2.34	1.93	1.02	2.01
Hf	1 ppm	2	2	3	4
Mo	5 ppm	15	<5	<5	<5
Na	0.05 ppm	17000	22000	11000	13000
Ni	200 ppm	<200	<200	<200	<200
Sb	0.2 ppm	0.4	<0.2	<0.2	<0.2
Sc	0.1 ppm	8.6	7.0	2.4	2.8
Se	5 ppm	<5	<5	<5	<5
Ta	1 ppm	<1	<1	<1	<1
Th	0.5 ppm	2.9	3.3	4.2	5.4
U	0.5 ppm	3.2	2.6	0.9	0.8
W	4 ppm	<4	<4	<4	<4
Zn	50 ppm	120	50	60	<50
La	1 ppm	15	14	16	18
Lu	0.1 ppm	0.2	0.2	0.1	0.1

* The top layer is mainly peat moss and organic material.

** The soil horizons were L-H over A_c over B_{m,f} over C.

different geochemical signatures. AR has higher concentrations of Ca and Co, whereas GL has higher Rb in pollen. The gold content was higher at AR than at GL. This finding confirms our original expectations.

The majority of elements analyzed show an increase in the upper concentration range from pollen at the AR site (Table 3). Only the elements Mo, Rb, Se and Th showed higher concentrations in pollen from the GL site.

In order to obtain a measure of precision in the neutron activation analysis, several samples of pollen were split up into three subsamples and analyzed in triplicate (Table 4). Elements were chosen that had consistent readings above the detection limits. The relative standard deviation (RSD) ranged from 2-47% for the complete set. Only five values were greater than 30% RSD, while the remaining 24 were below this value. The RSD for the AR-2 composite pollen sample for Na could not be determined as only two data points were available. The overall average precision excluding the five high values was 12%.

It is known that the gold content in some plants shows a seasonal variation (Boyle, 1979; Dunn, 1985). Boyle (1979) has shown numerous types of plants that are suspected to accumulate gold. Examples of some are: grasses, fungi, mosses, wormwood shrubs, Spiraea, honeysuckle and water hyacinth. Gold tends to have the highest concentrations in the above ground parts specifically the leaves and seeds.

Table 3. Element Concentrations in Dried Bee Pollen (Determined by Neutron Activation Analysis).

Element	Detection Limits	Concentration Range	
		Gordon Lake Site n=10	Anglo Rouyn Site n=10
Ag	0.1 ppm	<0.2	<0.2
As	0.01 ppm	<0.01 - 0.07	<0.01 - 0.15
Au	0.1 ppb	0.2 - 1.5	<0.3 - 2.8
Ba	5 ppm	<5 - 12	<5 - 18
Br	0.01 ppm	0.6 - 4.0	0.7 - 8.3
Ca	0.01 %	0.04 - 0.19	0.09 - 0.37
Co	0.1 ppm	<0.1 - 0.3	0.2 - 1.5
Cr	0.1 ppm	<0.2 - 0.4	<0.3 - 2
Cs	0.01 ppm	<0.10 - 0.19	<0.09 - 0.19
Fe	0.005 %	0.007 - 0.026	0.009 - 0.119
Hf	0.1 ppm	<0.01	<0.01
Mo	0.05 ppm	<0.08 - 0.45	<0.05 - 0.41
Na	1 ppm	53 - 260	<1 - 540
Ni	5 ppm	<5.0	<5.0
Rb	1 ppm	18 - 41	6 - 26
Sb	0.005 ppm	<0.005 - 0.015	<0.005 - 0.015
Sc	0.01 ppm	0.01 - 0.09	<0.01 - 0.42
Se	0.1 ppm	0.1 - 1.5	<0.1
Sr	10 ppm	<10	<10
Ta	0.06 ppm	<0.14	<0.09 - 0.10
Th	0.1 ppm	<0.1 - 0.3	<0.1 - 0.1
U	0.01 ppm	<0.02 - 0.03	<0.02 - 0.05
W	0.05 ppm	<0.05	<0.05 - 0.11
Zn	2 ppm	25 - 48	26 - 49
La	0.01 ppm	0.08 - 0.59	0.01 - 0.73
Ce	0.1 ppm	<0.1 - 0.8	<0.02 - 1.4
Nd	1 ppm	<1 - 1	<1 - 1
Sm	0.001 ppm	0.007 - 0.061	<0.001 - 0.100
Eu	0.03 ppm	<0.07	<0.11
Yb	0.005 ppm	<0.008 - 0.035	<0.008 - 0.068
Lu	0.001 ppm	<0.001 - 0.006	<0.002 - 0.012
Ash*	%	2.5 - 2.9	4.6 - 5.5

* Based on three samples from each location.

Table 4. Precision of Elemental Analysis in Bee Pollen Samples (Neutron Activation Analysis) Pollen Identification/% Relative Standard Deviation (Number).

Element	PAR-2 Composite/ % RSD** (n)	GL-1 Composite/ % RSD (n)	GL-2 Composite/ % RSD (n)
Au	11 (3)	41 (3)	34 (3)
Br	11 (3)	2 (3)	10 (3)
Ca	47 (3)	5 (3)	10 (3)
Co	18 (3)	40 (3)	18 (3)
Fe	18 (3)	5 (3)	7 (3)
Na	---*	20 (3)	5 (3)
Rb	12 (3)	6 (3)	13 (3)
Zn	7 (3)	6 (3)	10 (3)
La	36 (3)	19 (3)	17 (3)
Sm	18 (3)	27 (3)	13 (3)

* Not enough samples for precision calculation.

** All calculations are based on the population standard deviation σ_n as opposed to the sample standard deviation σ_{n-1} .

Pollen seemed to be an ideal sampling medium as it is close to the seed development and easily collected with bees.

The variation over our collection period of selected elements from color separated pollen is displayed in Figures 2 to 11. The elements selected were consistently above the analytical detection limit. The period for the GL site collections was June 5-July 24, and for AR June 4-July 22, 1985 (Table 1). The gold concentration at GL peaked in late June whereas at AR the highest concentration was in early June, tapering off until the last collection. The majority of the elements displayed an increase in concentration as the season progressed and all were higher at AR compared to the GL site. The exception was Rb which displayed the reverse behavior. The Zn concentrations in pollen did not differentiate between the two sites. Gold concentrations in pollen may display a seasonal variation at AR but at GL the concentration was fairly flat across the four collection periods. The elements Br, Ca, Co, Fe, Na, La, and Sm showed increasing concentrations in pollen from June to July predominately at the AR site. The factors that may be responsible would be different pollen populations and higher elemental composition of soil from the AR site.

The forage range of bees (approximately 50 km²) compared to Dunn's (1984) areas of gold enrichment (<0.5-1 km²) in vegetation are substantially different, by a factor of 50-100 times. This leads to two factors of potential metal

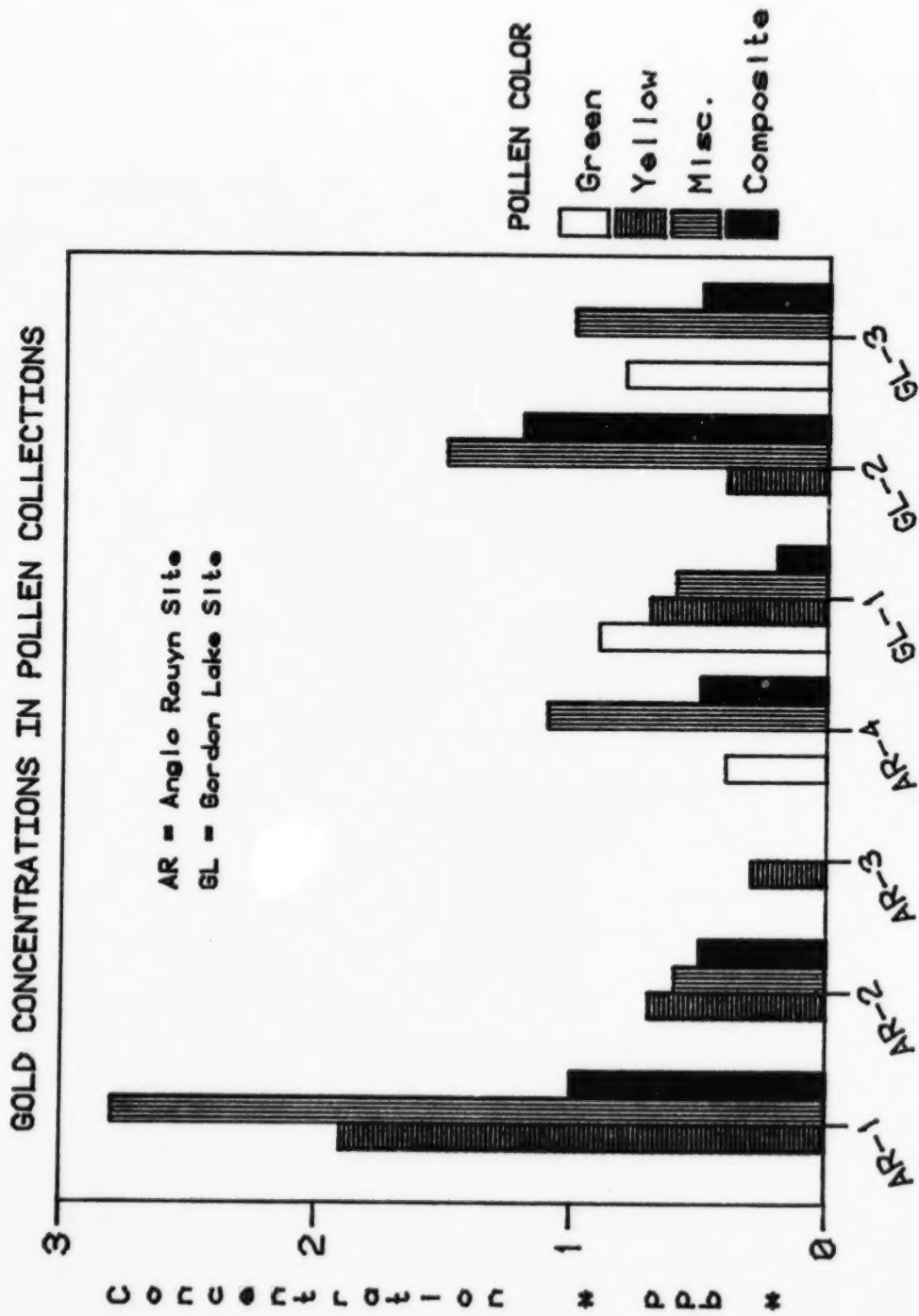


Figure 2.

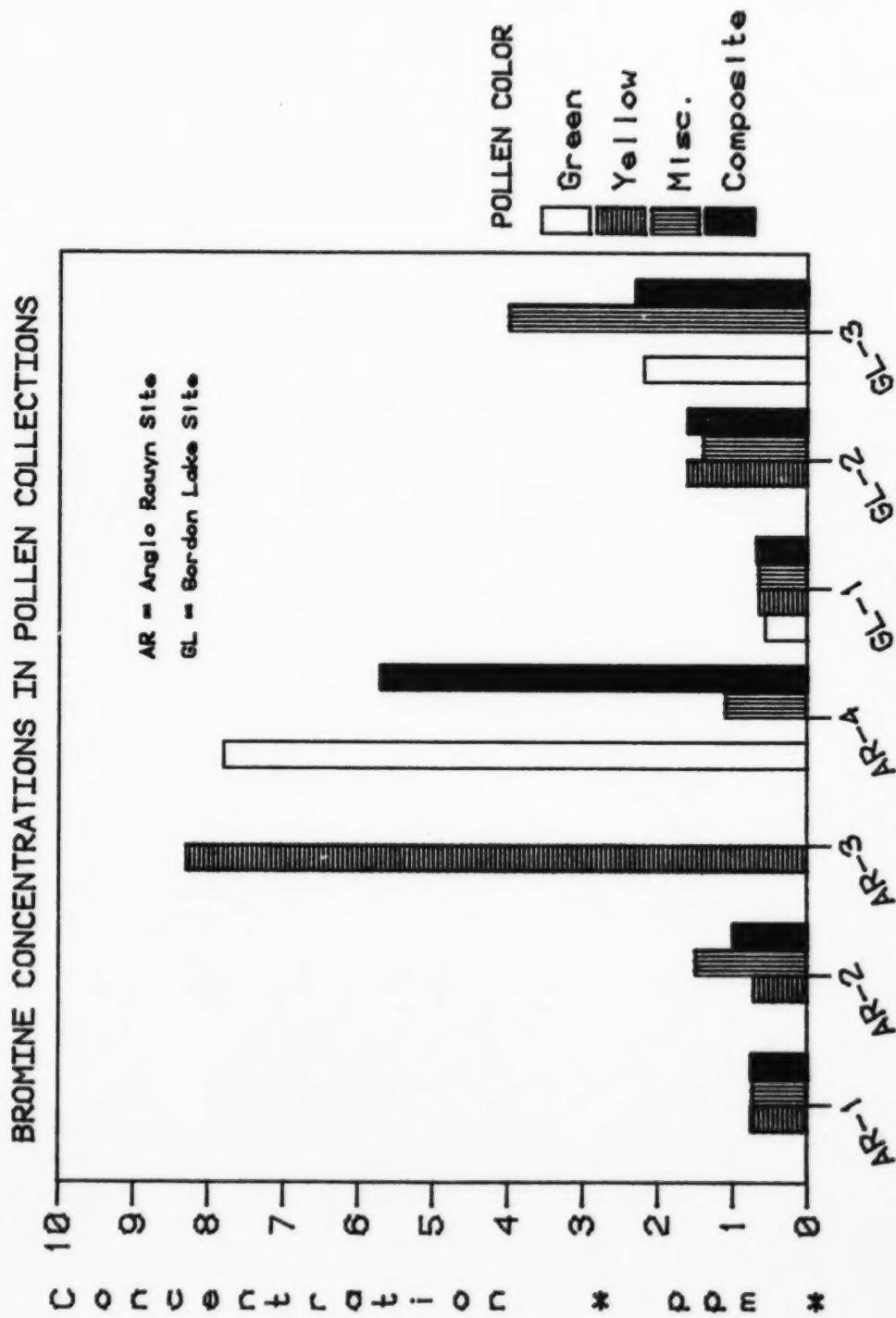


Figure 3.

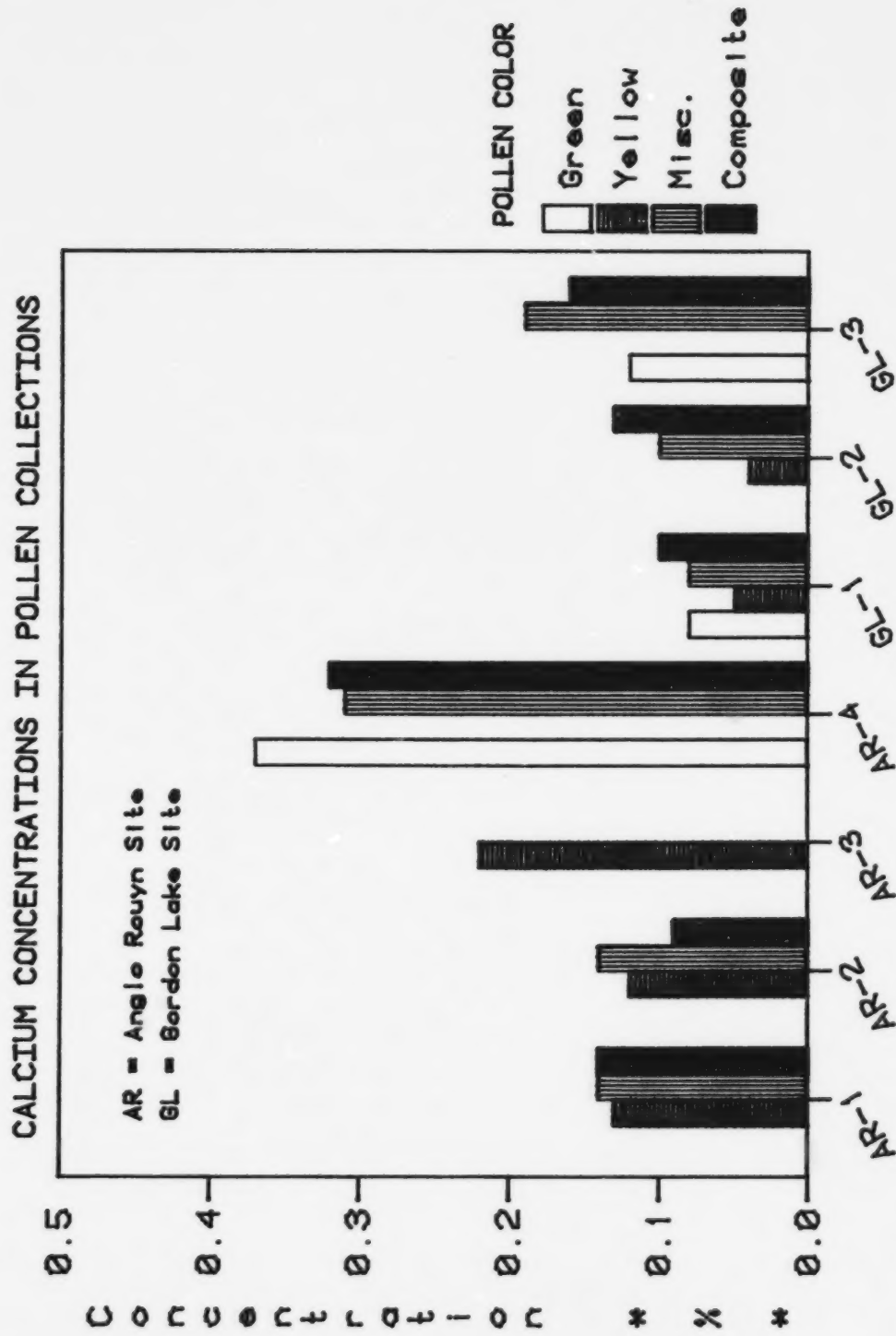


Figure 4.

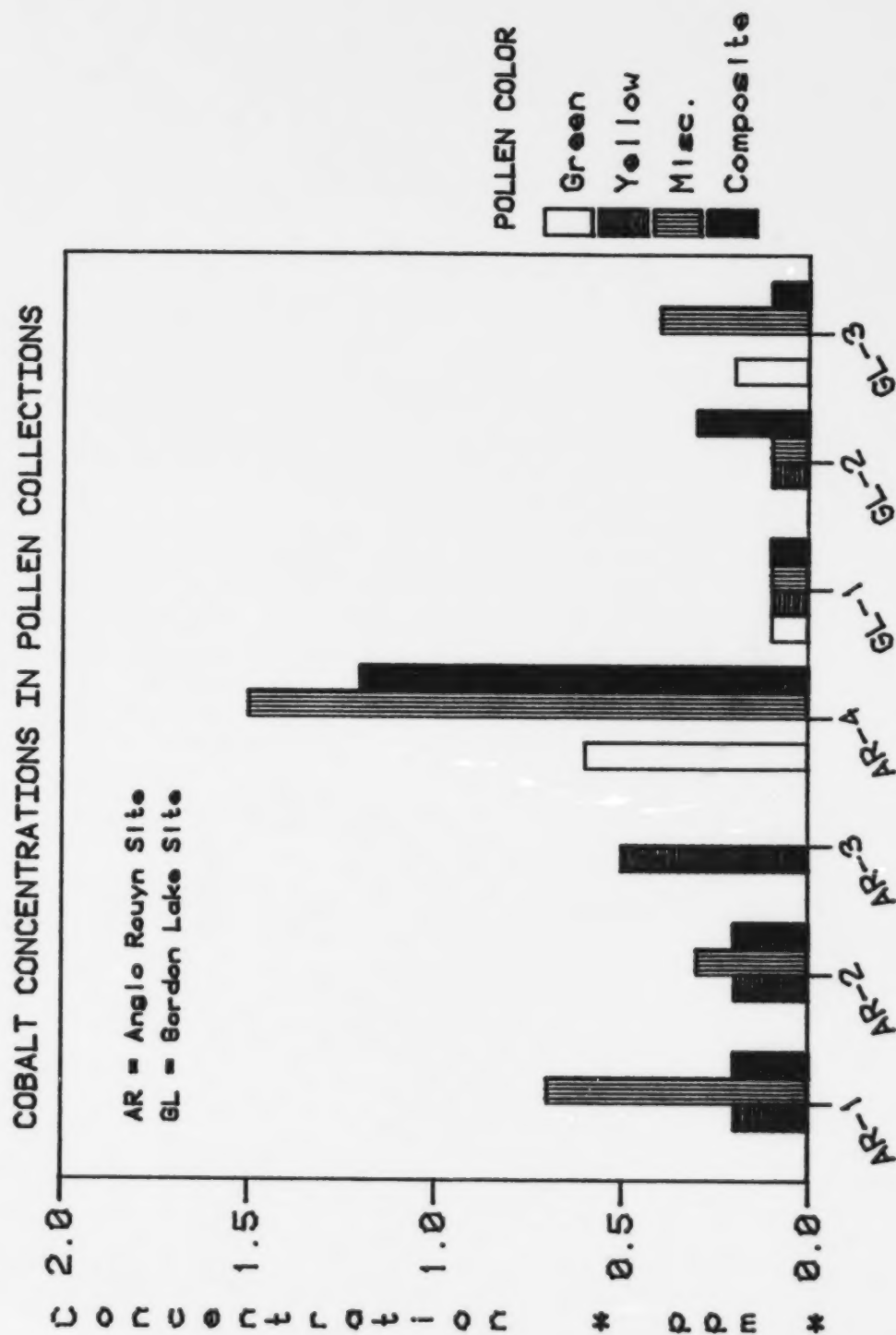


Figure 5.

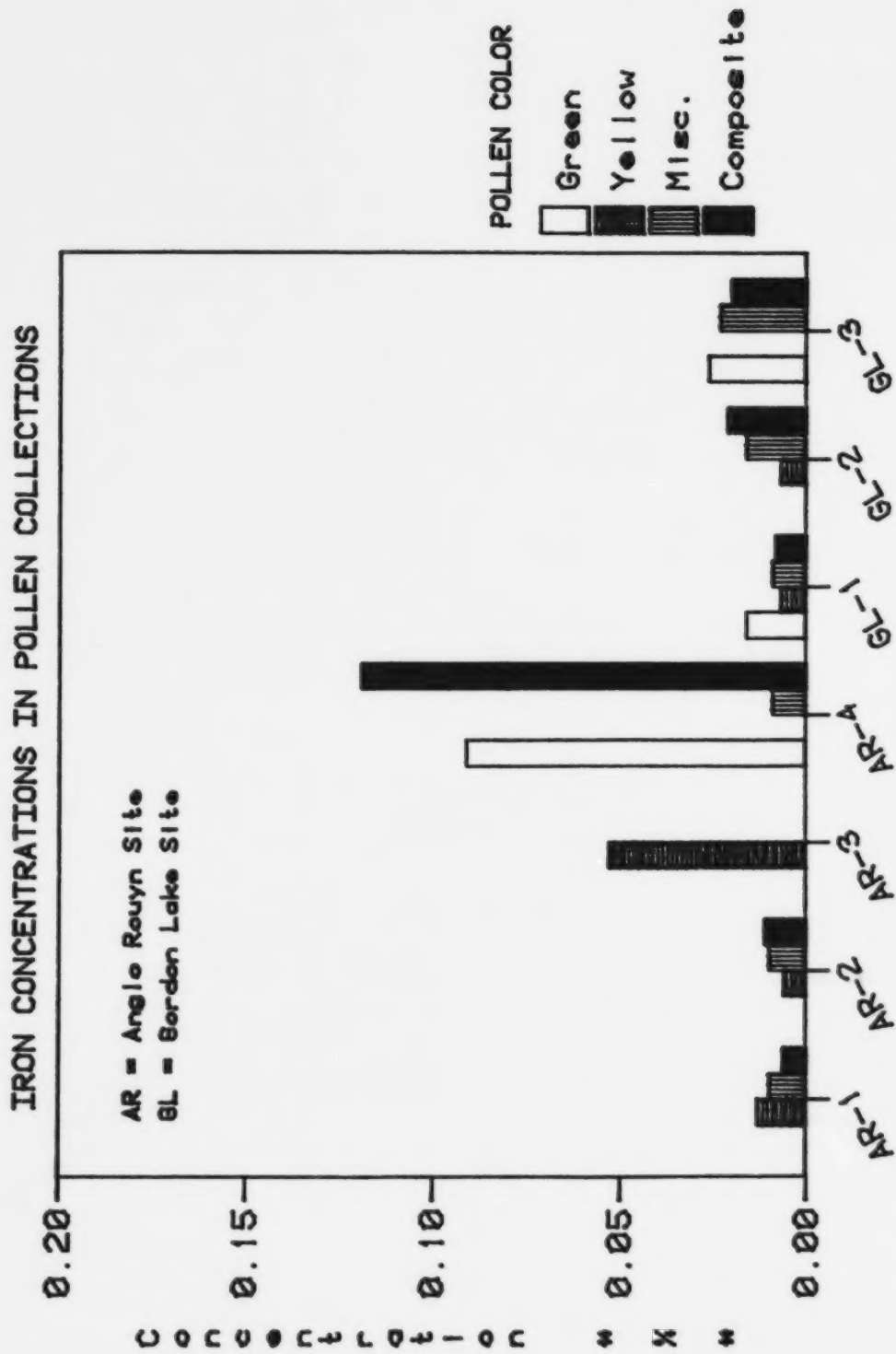


Figure 6.

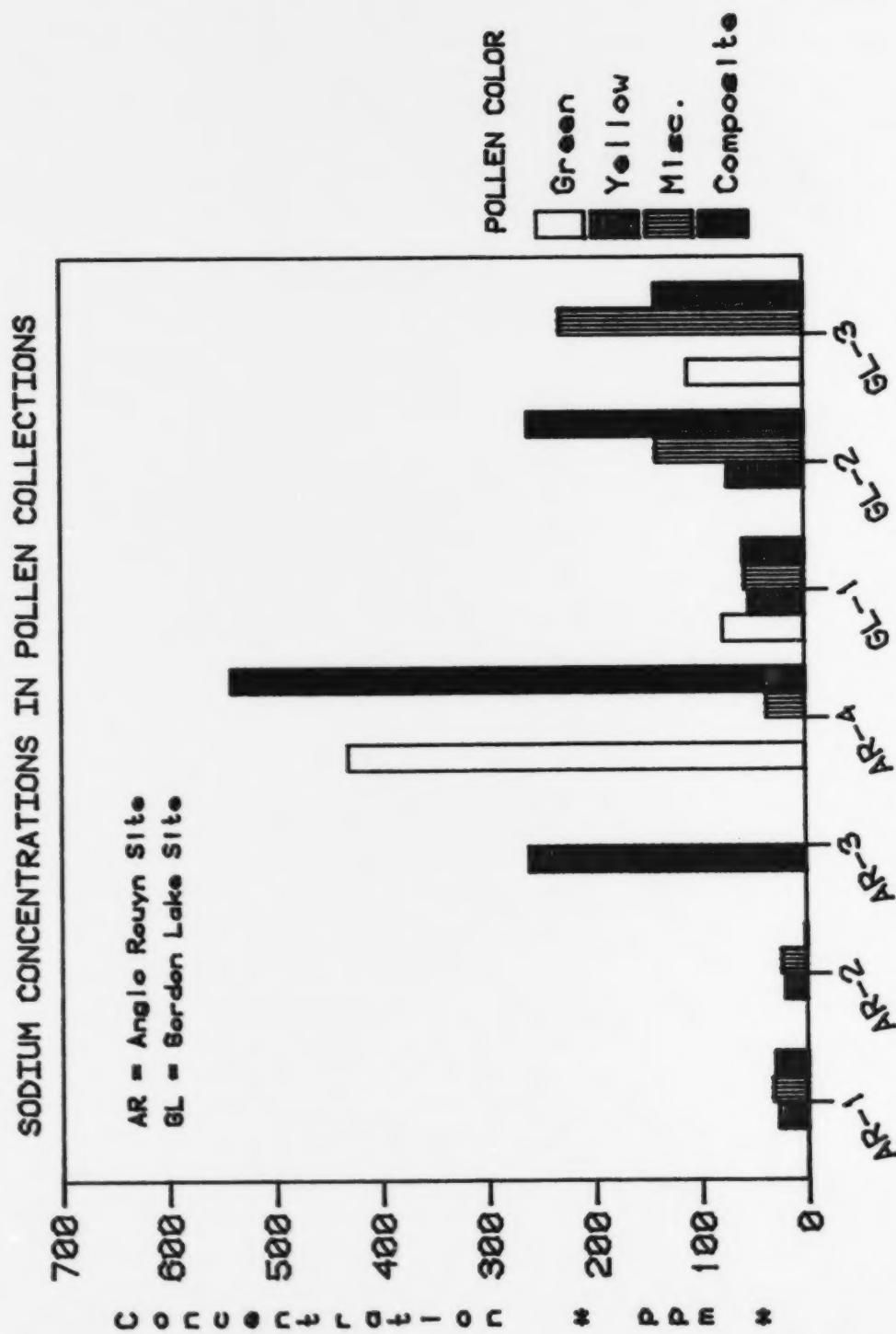


Figure 7.

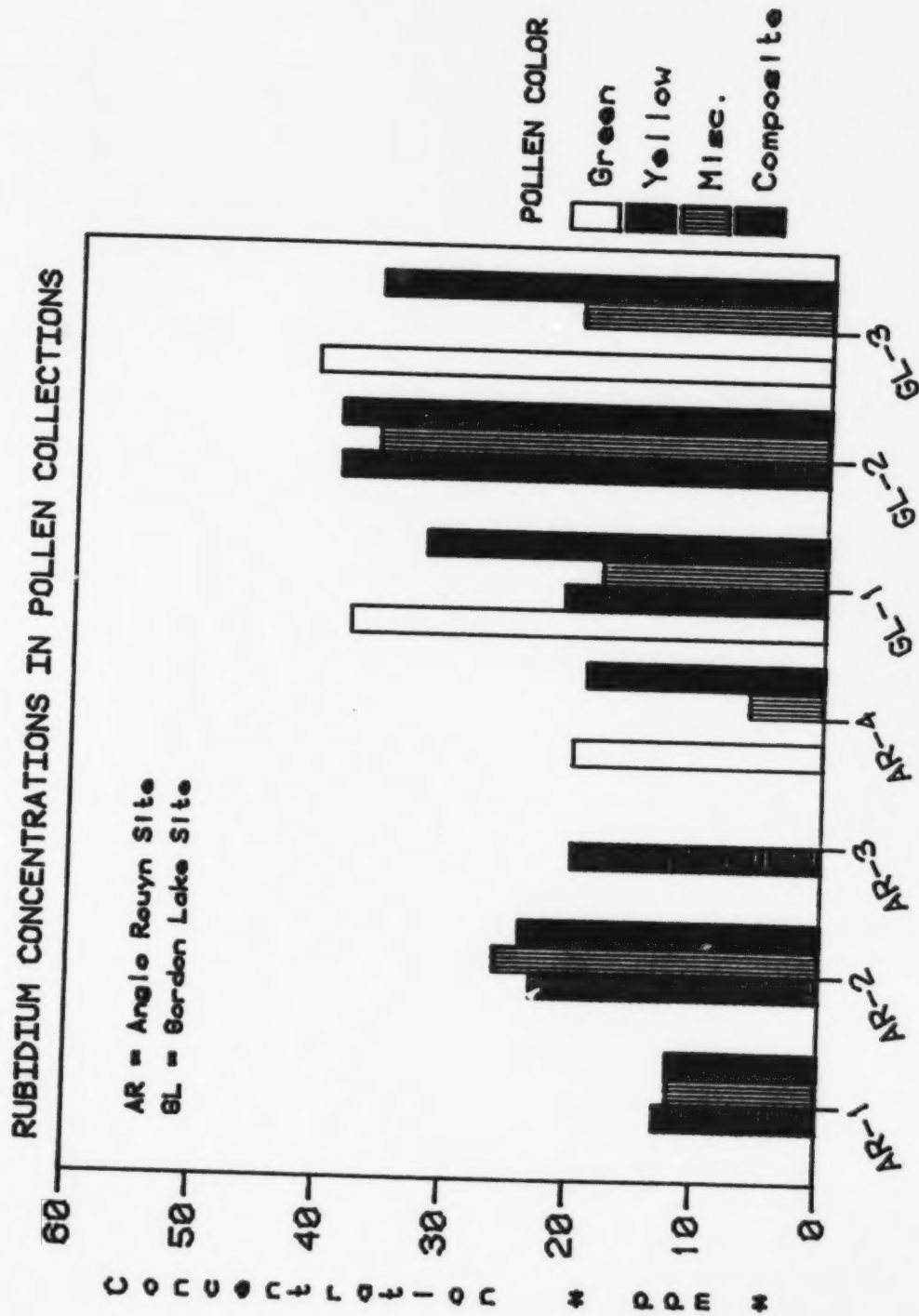


Figure 8.

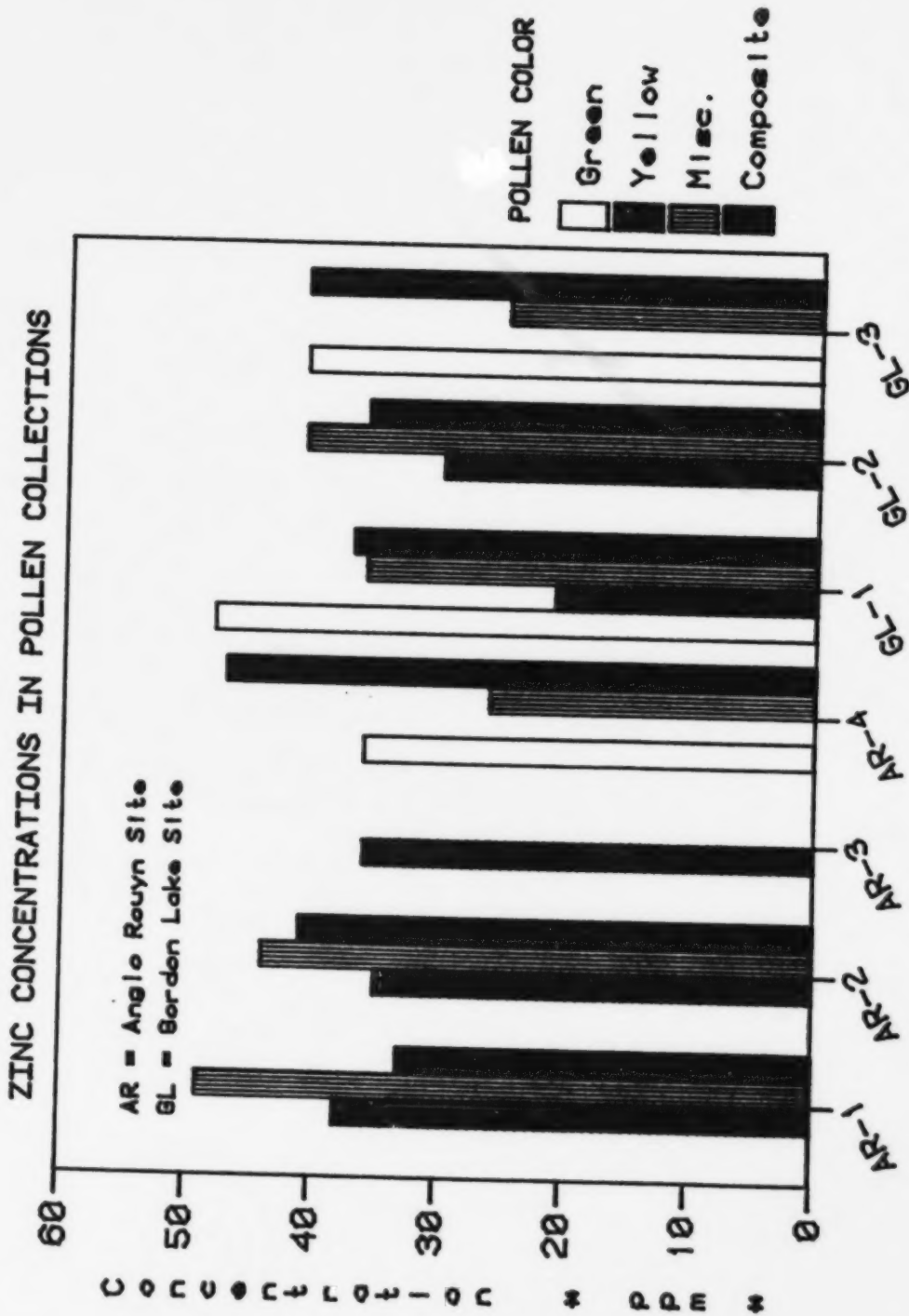


Figure 9.

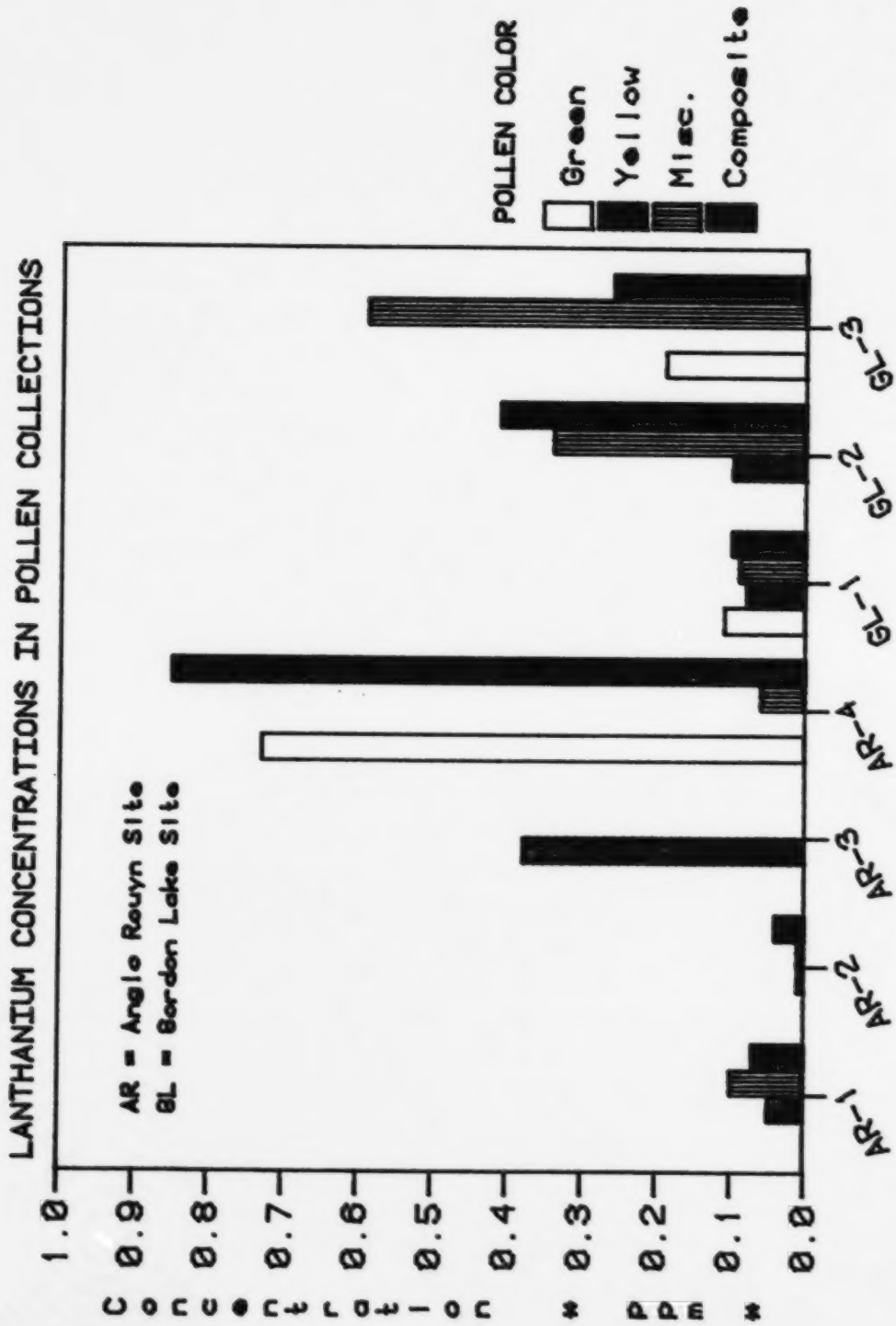


Figure 10.

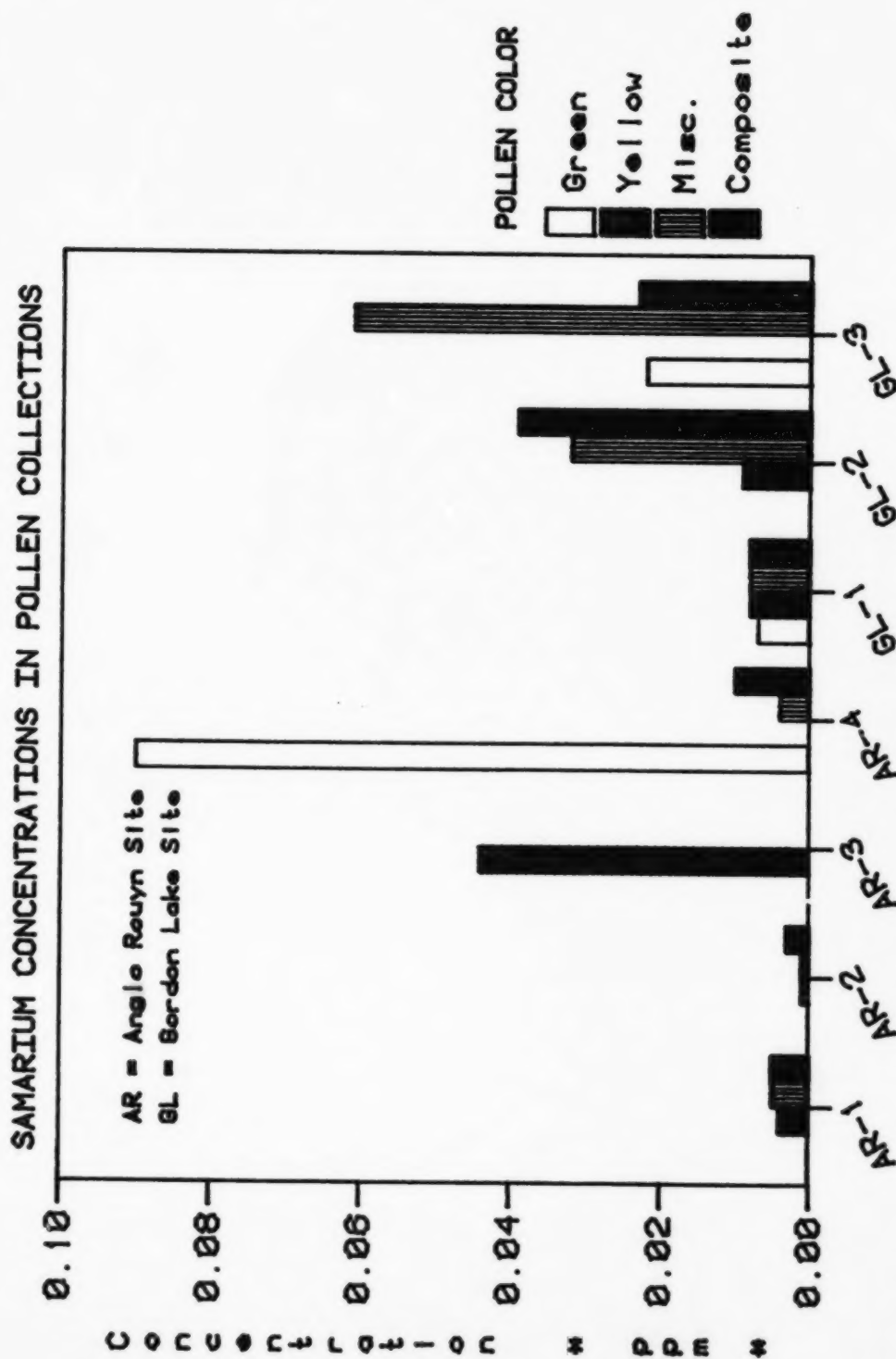


Figure 11.

dilution of anomalous element concentrations in bee pollen. Firstly, there was several square kilometres of gold enrichment in the area of the AR site as shown by alder pruning (Dunn, 1984). The evidence suggests some flowering species uptake gold as well as alders when results are expressed on an ashed basis. Secondly, not all of the flowering plants in the enriched zone would have the same capacity for gold and metal uptake. Dunn (1985) has shown by ashing whole plants that Labrador Tea (Ledum groenlandicum) gives gold concentrations of 2-4 times that of: Blueberry (Vaccinium spp.), Rose (Rosa acicularis), and Stiff Club Moss (Lycopodium annotinum). Table 5 contains a partial inventory of the plant species that were present in June, of which some were in the flowering stage. During our pollen collections Labrador Tea was blooming but only in the latter part of July. These two effects may mask the gold and metal concentrations from enriched zones.

Table 5. Plant Species Identified at Each Site* (June 1985).

Anglo Rouyn Site	Gordon Lake Site
Early blue violet (<u>Viola adunca</u>)	Fireweed (<u>Epilobium angustifolium</u>)
Bunchberry (<u>Cornus canadensis</u>)	Bearberry (<u>Arctostaphylos uva-ursi</u>)
Wild sarsaparilla (<u>Aralia nudicaulis</u>)	Pin cherry (<u>Prunus pensylvanica</u>)
Dandelion (<u>Taraxacum officinale</u>)	Blueberry (<u>Vaccinium myrtilloides</u>)
Labrador Tea (<u>Ledum groenlandicum</u>)	Marsh cinquefoil (<u>Potentilla palustris</u>)
	Two leaved Solomon's seal (<u>Maianthemum canadenses</u>)
	Red currant (<u>Ribes triste</u>)
	Bastard toad flax (<u>Comandra umbellate</u>)

* This is by no means an exhaustive list of plant species, only as an indicator of variety and types of species honey bees would visit.

DISCUSSION

The original interest of our preliminary work with bees as a biogeochemical indicator for metals in northern Saskatchewan was to evaluate the pollen collected from the two sites. The results from pollen collections confirm our original expectations of higher metal concentrations from pollen in plants grown over mineralized soil. The metal content of soil reflects the same results. It must be pointed out that other investigators (Dunn, 1985) have found anomalous biogeochemical zones where no mineralization was expected. This may explain the two higher gold readings found from the GL site.

Free et al. (1983) points out the uptake of an element is a function of soil acidity, organic content, interactions with other elements and the growth stage of the plant. It is known that different plant species growing close together can show great differences in elemental composition (Warren, 1960). The surface geology of the area around the GL site consists of glaciofluvial pockets of sand and gravel surrounded by sand and till areas. The AR site has more topographic variation, and is characterized by rock outcroppings, rock concealed by a thin moraine and glaciolacustrine deposits. The comparison of our two sites would be easier if two areas of similar surface geology were chosen. We do know that some flowering species absorb gold more readily than others (Dunn, 1985). The large forage range of bees

(50 km²) compared to the areas of anomalous gold in vegetation (0.5-1.0 km²) results in a dilution of the metal concentration in pollen. Our pollen results for gold if expressed on an ash weight basis would be comparable to values Dunn (1985) found in ashed alder twigs even after the above-mentioned dilutions. The uptake of gold by the flowering species would be comparable to that found in alder twigs. The results show that the metal content of pollen varies moderately within two geographic regions of northern Saskatchewan. There were insufficient data to perform a complete statistical evaluation of the metals in pollen or soil. Based on our data it appears that data precision goals of 15-30% RSD are achievable given the diverse nature of the sample. As well, there may be some seasonal variation of various elements in pollen collected by bees over the period June-July 1985, although more samples taken over a longer period of time would confirm this.

Free et al. (1983) found that hives at the same location and time gave quite different metal concentrations in the pollen especially for iron and zinc. The correspondence analysis indicates the variability within each site is as important as that between sites.

Boyle (1979) reports conflicting evidence of direct gold uptake from soil to plants, he states "this ratio depends on a veritable host of factors, and one would expect wide variations and reversals to be general."

Airborne dust could contribute to higher metal concentrations in pollen at GL. The traffic along the Key Lake road produced a significant amount of dust at the GL site. A fraction of the small (<10 micron) dust particles may become associated with the anther of the flower resulting in pollen contamination, especially if the dust was high in metal content. Further investigations should address airborne dust and pollen contamination.

The underlying process at play in using bee pollen as a tool for locating ore bodies is quite complex. The present study has shown that pollen can be used as a potential indicator for areas of increased mineralization.

RECOMMENDATIONS FOR FUTURE STUDIES

1. Bee pollen should be collected from agricultural areas for comparison of metal levels and to aid in the determination of a good control sample. As well the additional samples would provide a larger data base for statistical comparisons.

2. More frequent collections and an extended collection period from mid-June to late August are recommended. Color separation of pollen did not yield any further insights into seasonal variations, which may be due to the limited number of pollen colors and the relative abundance of each color.

3. More hives should be established at a particular location to investigate the extent of interhive differences.

4. Plant species in a particular forage range should be catalogued. Flowering species should be analyzed for metal content at different stages in their growth.

5. Hives should be located over areas of similar surface geology both in mineralized and nonmineralized zones.

6. The hives should be located far enough from roads so that airborne dust would not contaminate the pollen. The chemical composition of airborne dust should be investigated in order to assess its elemental contribution to pollen.

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